Enabling Rapid and High-confidence Metabolite Identification using HILIC-QTOF based MS/MS-RT library

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Overview

- A metabolite MS/MS-RT library (Bruker HMDB Metabolite Library 2.0) and a comprehensive metabolomics workflow (Bruker T-ReX LC-QTOF solution) were developed to achieve high-confidence and rapid metabolite identification in metabolomics studies based on matching accurate precursor mass, isotopic pattern, retention time (RT) and MS/MS fragment spectral information.
- In this work, the MS/MS-RT library was expanded by introducing HILIC based RTs for more comprehensive and high-confidence metabolite identification.
- Detailed investigation was carried out to optimize the parameters for HILIC-MS acquisition, including mobile phase buffers and the LC gradient. Retention time normalization techniques successfully correct RT shifts caused by various experimental condition changes.
- We also demonstrated the workflow using the HILIC MS/MS-RT library to perform identification in a different and independent laboratory.

Introduction

- LC-MS has become a leading platform for metabolomics, with the capability of capturing thousands of metabolite features from biological samples. Metabolite identification is a crucial step to link the detected features with biological process. To enable high-confidence and rapid identification, we previously constructed a MS/MS and retention time (RT) library based on reversed phase (RP) LC-QTOF system. The identification solution was demonstrated to be able to provide useful information for metabolomics studies.

- Hydrophilic interaction LC (HILIC), as a valuable complimentary technique to RPLC, has been widely used to improve metabolite coverage. In this work, we focus on expanding our RP library by introducing the HILIC separation. We also demonstrate the workflow using the HILIC MS/MS-RT library to perform identification.

Library Construction

Instruments Setup

- High resolution Impact II HD Q-TOF MS (Bruker Daltonics)
- Elute UHPLC system (Bruker Daltonics)
- HILIC column (1.9 µm, 100×2.1 mm) (Bruker Daltonics)

Metabolite Standards

- 829 human endogenous metabolites from HMDB

Library Construction (cont.)

Mobile Phase Buffer Optimization

- Considering HILIC may not be as stable as RPLC separation, we carefully optimized the mobile phase buffer to tightly control the running conditions. After the analysis of 35 amino acids, aromatic compounds and carbohydrates, we found 10 mM NH4Ac+0.1% FA to be the optimal buffer for the mobile phase, in which most of the metabolites were detected as a reproducible peak with good peak shape.

#	Mobile phase buffer	pH
1	0.1% FA	2.7
2	10 mM NH_4Ac	6.4
3	10 mM NH_4FA	5.7
4	10 mM NH_4Ac, 0.1% FA	3.6
5	10 mM NH_4FA, 0.1% FA	3.3

MPA: water;
MPB: 95%/5% ACN/water (v/v)
Gradient: 0 min: 100% B; 2 min: 100% B; 17 min: 50% B; 20min: 50% B
Flow rate: 0.25 mL/min

Compound	SD of Retention Time (s) FA	NH4Ac	NH4FA	NH4Ac+FA	NH4FA+FA	RSD of Peak Width FA	NH4Ac	NH4FA	NH4Ac+FA	NH4FA+FA
Dimethylglycine	8.0	1.8	0.2	1.6	0.4	18.05%	9.40%	0.80%	2.76%	3.68%
gamma-Aminobutyric acid	10.2	1.3	1.3	1.0	0.4	21.97%	7.20%	5.47%	4.09%	3.68%
L-Isoleucine	23.8	1.4	0.1	1.2	0.4	16.75%	4.92%	2.35%	1.03%	1.83%
L-Histidine	1.3	11.1	4.8	1.6	1.8	4.66%	26.06%	31.73%	4.11%	1.36%
3-Methylhistidine	0.1	0.1	2.7	0.4	0.5	4.58%	2.66%	9.70%	2.41%	0.83%
Kynurenine	1.3	1.2	0.4	1.0	0.4	6.90%	5.56%	2.54%	0.36%	2.08%
L-Aspartyl-L-phenylalanine	0.4	0.4	0.5	0.1	0.7	0.62%	2.82%	6.18%	4.94%	0.42%
Glycyl-L-leucine	0.6	0.7	0.2	1.1	0.5	3.76%	2.49%	0.96%	2.61%	1.00%
N-Acetyl-L-alanine	4.8	5.5	0.2	1.9	0.7	4.62%	2.30%	0.93%	3.36%	3.04%
Quinaldic acid	4.7	0.6	0.9	0.7	9.3	0.78%	7.84%	4.89%	2.80%	0.90%
Methylhippuric acid	0.6	0.5	0.2	0.3	0.6	5.62%	3.20%	0.47%	2.20%	2.67%
Biopterin	0.9	0.8	1.7	0.4	0.5	2.34%	0.29%	1.58%	3.85%	3.12%
Hematoporphyrin	0.2	1.2	1.3	0.3	0.5	0.24%	5.85%	0.65%	3.72%	5.01%
p-Aminobenzoic acid	0.2	0.7	0.0	0.2	0.2	0.89%	8.43%	10.06%	1.22%	2.59%
Famotidine	2.0	0.8	12.2	10.3	11.0	8.39%	12.24%	1.51%	5.99%	16.66%
Clotrimazole	0.6	0.2	0.4	0.7	0.3	0.87%	0.71%	2.38%	1.56%	0.92%
Levofloxacin	1.3	1.8	6.2	0.1	6.6	7.33%	2.14%	3.04%	6.15%	2.17%
Triamterene	6.1	5.9	26.0	5.9	16.7	10.45%	1.04%	7.04%	6.33%	0.57%
Phenylpropanolamine	2.0	1.6	5.3	1.6	12.0	6.01%	9.76%	21.28%	9.76%	18.38%
Pseudoephedrine	8.1	4.3	18.8	6.4	18.6	13.30%	11.13%	32.11%	1.92%	10.44%
Lactose	1.4	1.4	1.6	0.2	0.7	3.77%	7.27%	3.23%	1.45%	3.77%
N-Acetyl-D-glucosamine	1.1	0.8	1.3	1.1	0.9	2.39%	0.88%	1.26%	3.26%	2.06%
Glucosamine 6-sulfate	0.1	1.0	1.0	0.4	0.8	3.91%	3.31%	2.59%	2.30%	1.33%
Sialyllactose	0.0	0.1	0.8	0.2	0.4	2.34%	0.59%	0.40%	0.45%	1.05%

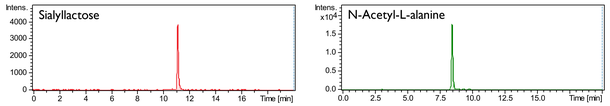


Figure 1. HILIC mobile phase buffer optimization. EICs for two examples used for gradient optimization.

Gradient Optimization

- To further improve the performance of HILIC method, we optimized the LC gradient.

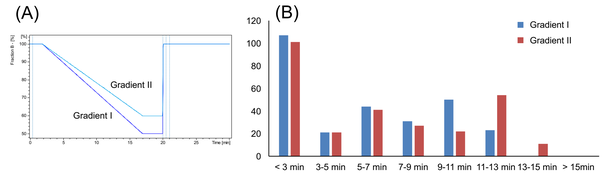
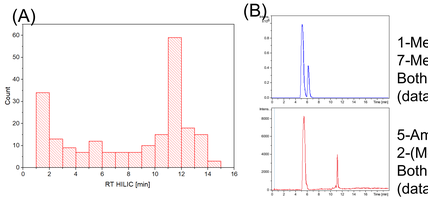


Figure 2. HILIC gradient optimization. (A) Gradient comparison: gradient I ends at 50%B and gradient II ends at 60%B. (B) RTs distribution of 270 compounds using two gradients.

Overview of HILIC MS/MS-RT Library

- 829 standards were injected in triplicates under both positive and negative ion mode. QC (i.e. RT calibrant) was injected every 9 samples to ensure stability of RT collection.
- Retention times for 634 metabolites were determined (standard deviation < 6 sec).
- All RTs were normalized to the same “RT calibrant” (see below).



1-Methylguanine &
7-Methylguanine
Both RT are 1.42 min in RPLC
(data not shown)

5-Aminopentanoic acid &
2-(Methylamino)isobutyric acid
Both RT are 1.26 min in RPLC
(data not shown)

Figure 3. (A) RTs in HILIC MS/MS-RT Library. (B) Two examples for HILIC separation of two metabolite pairs that show the same m/z and RT in RP separation between 0 to 2 min (i.e. the typical RP void volume).

Inter-lab Transfer of RTs

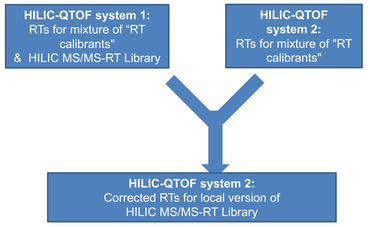


Figure 4. Workflow for RT correction

RT information can significantly increase the confidence in metabolite identification. However, since the RT of HILIC separation can be readily affected by minor variations in experimental conditions, a multipoint RT calibration method was used to correct RT shift between the library RTs and experimental RTs. This was shown to be effective to overcome various experimental variations, including using different LC-MS systems.

RTs for the “RT calibrants” and the Library were determined using system 1 (in Alberta, Canada). For system 2, the RTs for the "RT calibrants" were determined experimentally (in Bremen, Germany). The RTs in the HILIC MS/MS-RT Library for system 2 were then corrected using a multipoint RT calibration algorithm .

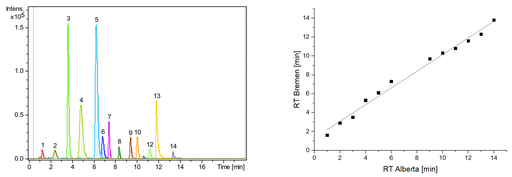


Figure 5. Inter-lab transfer of RTs. (A) Extracted ion chromatograms of “RT calibrants”. (B) Multi-point linear regression of calibrants RT for both systems.

Validation in Real Samples

Urine samples were prepared by diluting filtered urine with three times of acetonitrile to match the sample composition close to the initial mobile phase. HILIC-MS/MS data acquired on system 2 (in Bremen, Germany) were used for metabolite identification. Both positive ion mode data and negative ion mode data were collected. Very reproducible LC-MS data across replicates were generated as shown below.

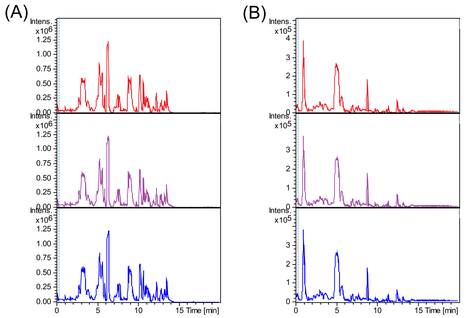


Figure 6. Base peak chromatograms of urine sample with triplicate injection in (A) positive ion mode and (B) negative ion mode.

The number of identified features was determined by applying the RT correction workflow shown in Figure 4. The annotation was conducted using the MetaboScape software (Bruker Daltonics) by automatically matching precursor mass, precursor isotopic pattern (mSigma value), corrected retention time, as well as fragment spectrum information.
130 metabolites were identified in positive ion mode and 98 metabolites were identified in negative ion mode.

Conclusions

1. A high quality HILIC based MS/MS-RT library was constructed. The current library contains more than 600 relevant human endogenous metabolites.
2. By using this library in combination with the MetaboScape software, a fully integrated workflow to perform rapid and high-confidence metabolite identification based on HILIC separation is enabled.
3. By applying the established RT correction strategy the inter-lab portability of this identification approach could be demonstrated.

Acknowledgements

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